Recovery of the immune system after exercise

Peake, Jonathan M; Neubauer, Oliver; Walsh, Neil P; Simpson, Richard J

Journal of Applied Physiology

DOI:
10.1152/japplphysiol.00622.2016

Published: 01/05/2017

Peer reviewed version

Recovery of the immune system after exercise

Jonathan M. Peake[1,2], Oliver Neubauer[1], Neil P. Walsh[3], Richard J. Simpson[4]

[1] School of Biomedical Sciences and Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia.

[2] Centre of Excellence for Applied Sport Science Research, Queensland Academy of Sport, Brisbane, Australia.

[3] Extremes Research Group, School of Sport, Health and Exercise Sciences, Bangor University, Bangor, UK.

[4] Laboratory of Integrated Physiology, Department of Health and Human Performance, University of Houston, Houston, Texas, USA.

Running head: Immune system recovery after exercise

Word count in main document: 5,253

Corresponding author:
Jonathan Peake
Institute of Health and Biomedical Innovation
Queensland University of Technology
Kelvin Grove, QLD 4059
Australia
Email: jonathan.peake@qut.edu.au
Phone: +61 7 3138 6140

ABSTRACT

The notion that prolonged, intense exercise causes an 'open window' of immunodepression during recovery after exercise is well accepted. Repeated exercise bouts or intensified training without sufficient recovery may increase the risk of illness. However, except for salivary IgA, clear and consistent markers of this immunodepression remain elusive. Exercise increases circulating neutrophil and monocyte counts, and reduces circulating lymphocyte count during recovery. This lymphopenia results from preferential egress of lymphocyte subtypes with potent effector functions (e.g., NK cells, $\gamma\delta$ T cells and $CD8^+$ T cells). These lymphocytes most likely translocate to peripheral sites of potential antigen encounter (e.g., lungs, gut). This redeployment of effector lymphocytes is an integral part of the physiological stress response to exercise. Current knowledge about changes in immune function during recovery from exercise is derived from assessment at the cell population level of isolated cells ex vivo or in blood. This assessment can be biased by large changes in the distribution of immune cells between blood and peripheral tissues during and after exercise. Some evidence suggests that reduced immune cell function in vitro may coincide with changes in vivo and rates of illness after exercise, but more work is required to substantiate this notion. Among the various nutritional strategies and physical therapies that athletes use to recover from exercise, carbohydrate supplementation is the most effective for minimizing immune disturbances during exercise recovery. Sleep is an important aspect of recovery, but more research is needed to determine how sleep disruption influences the immune system of athletes.

Keywords: open window; repeated exercise bouts; immunodepression; overreaching; sleep

INTRODUCTION

The immune system is integral to the body's defense against infection. It also influences other physiological systems and processes, including tissue repair, metabolism, thermoregulation, sleep/fatigue, and mental health. Over the past 40 years, exercise immunology has developed into its own discipline based on the recognition that the immune system mediates many exercise effects and that stress responses mediated through the nervous and endocrine systems play a key role in determining exercise-induced immune changes (84). A classic paradigm in exercise immunology is that an 'open window' of immunodepression can occur during recovery from intense exercise. In particular, this paradigm proposes that after intense exercise, some immune variables (e.g., lymphocyte and natural killer cell numbers, antibody production) transiently decrease below preexercise levels. As a result of this immunodepression microbial agents, especially viruses, may invade the host or reactivate from a latent state, leading to infection and illness (87). If exercise is repeated again while the immune system is still depressed, this could lead to a greater degree of immunodepression and potentially a longer window of opportunity for infection (87).

Exercise-induced fatigue exists on a continuum. Repeated bouts of intense exercise on the same day or over several days may cause acute fatigue, as indicated by an inability to maintain exercise workloads (64). An athlete who trains intensely for 1–2 weeks may experience a state of 'functional overreaching', which is associated with a temporary performance decrement, followed by improved performance. Intense training over an extended period without sufficient balance between training and recovery may lead to 'nonfunctional overreaching' (NFOR) (64). This condition is typically characterized by persistent fatigue, performance decrement, muscle soreness, and psychological and hormonal disturbances that can last for

weeks or months. Depending on the time needed to recover from NFOR, an athlete may be diagnosed (retrospectively) as experiencing 'overtraining syndrome' (64).

Recognition of the link between excessive training and risk of illness has stimulated interest in nutritional supplements and physical therapies to counteract immunodepression and to restore immune function after exercise training. In this mini-review, we update the current state of knowledge about the temporal changes in the immune system following exercise; how repeated bouts of exercise on the same day, extended periods of intense training, and sleep disruption influence the immune system; and the efficacy of various strategies for restoring immune function after exercise.

LEUKOCYTE REDEPLOYMENT DURING EXERCISE AND RECOVERY

A single exercise bout causes profound changes in the number and composition of blood leukocytes that may persist long into exercise recovery. All major leukocyte subpopulations tend to increase in number during exercise as a result of hemodynamic shear stress and/or catecholamines acting on leukocyte β_2 adrenergic receptors (126). The postexercise recovery period is marked by opposite effects on blood neutrophil and lymphocyte numbers. Neutrophil number (and, consequently, the total leukocyte count) often continues to increase long into the recovery period (up to 6 h after exercise cessation), particularly if the exercise bout is prolonged (>2 h) (86). This sustained 'neutrophilia' is characterized by an increased presence of immature, less differentiated, precursor neutrophils in the blood (117), most likely in response to the increased plasma levels of soluble agents including glucocorticoids, growth hormone, and cytokines such as IL-6 and granulocyte colony-stimulating factor, which

mobilize myeloid cells from the bone marrow (117). Although this neutrophilia following prolonged exercise is akin to that observed during bacterial infection (>7.0 $\times$ 10^6/ml), 24 h of recovery is usually sufficient for neutrophil number to return to normal (126). A delayed monocytosis is sometimes observed within 1–2 h after very prolonged exercise, but monocyte number typically returns to the resting level within 6 h after exercise cessation (126).

By contrast, lymphocyte number decreases rapidly after exercise. Following prolonged and/or high-intensity exercise in particular, lymphocyte number commonly decreases to below the preexercise value within as little as 30 min (126). This 'lymphopenia' can often reach levels typical of clinical lymphopenia (<1.0 $\times$ 10^6/ml) but the lymphocyte count is usually restored to both the resting and clinically normal level within 4–6 h of recovery (126). After prolonged bouts of exercise (e.g., 2 h cycling), natural killer (NK) cells (which account for most of the exercise-induced lymphocytosis) may be ~40% lower than the baseline value for up to 7 d after exercise (104). Exercise-induced lymphopenia reflects the preferential movement of lymphocyte subtypes with potent effector functions (e.g., NK cells, $\gamma\delta$ T cells, and $CD8^+$ T cells) out of the blood. Even within these subsets, there is a preferential egress of discrete subtypes of highly differentiated NK-cells, $\gamma\delta$ T cells, and $CD8^+$ T cells with phenotypes associated with tissue-migrating potential, and effector capabilities (107).

The rapid lymphopenia observed during the early stage of exercise recovery was initially of concern, particularly because early studies reported large rates of lymphocyte apoptosis (programmed cell death) after exhaustive exercise (62). However, these findings have not been substantiated. Subsequent studies have reported lymphocyte apoptosis in the order of 0–2% after exercise, even though the blood lymphocyte count was up to 30–40% lower than at rest (66, 105). Lymphocytes and monocytes leave the blood in large numbers during

exercise recovery under the influence of glucocorticoids. Lymphocyte subtypes that preferentially egress the peripheral blood during exercise recovery also have phenotypes consistent with tissue migration (e.g., expression of surface adhesion molecules, chemokine receptors) (108). These lymphocytes most likely translocate to peripheral sites of potential antigen encounter, such as the lungs or the gut (48).

The skin has long been considered a likely destination for effector lymphocytes in response to exercise and stress in general (24). However, recent evidence indicates that $CD8^+$ T cells and NK cells mobilized by exercise do not express cutaneous homing receptors on their surface (121). Exercise appears to 'prime' effector T cells, thereby allow them to transmigrate to the peripheral tissues that require enhanced immune surveillance following physical stress (54). Compared to the resting condition, the percentage of circulating lymphocytes expressing effector cytokines is lower following prolonged exercise (115), but it is unknown whether this decline reflects impairment at the individual cell level or preferential movement of effector T cells into peripheral tissues (e.g., lungs, gut). Recent evidence showing that exercise redeploys T cells that are specific to latent herpesviruses such as cytomegalovirus (CMV) and Epstein–Barr virus (EBV) (111, 112) suggests that this response may be a countermeasure against stress-induced viral reactivation (107). Exercise may also mobilize 'older' functionally exhausted/senescent lymphocytes to undergo apoptosis in the tissues and allow new 'recruits' to take their place (106, 107).

Monocytes mobilized by exercise are likely to infiltrate skeletal muscle and differentiate into tissue-resident macrophages that facilitate repair and regeneration, particular following arduous bouts of exercise that cause significant skeletal muscle damage (85). Monocytes with effector phenotypes are also preferentially redeployed after exercise. The $CD14^+/CD16^+$

'proinflammatory' monocytes are preferentially mobilized over their $CD14^+/CD16^-$ counterparts (109). Monocyte expression of pathogen recognition receptors (e.g., toll-like receptors [TLRs]) tends to decrease in response to moderate-intensity exercise (109). Conversely, prolonged, intense exercise (60 km cycling time trial) increases TLR2 and TLR4 expression on monocytes, which may indicate a heightened proinflammatory state (11). A recent study showed that acute exercise mobilizes angiogenic T cells, which may facilitate vascular remodeling during exercise recovery (53). Exercise is also known to mobilize hematopoietic stem cells, which may participate in skeletal muscle repair and regeneration after exercise (25, 49). It has been suggested that exercise may have a role as an adjuvant to mobilize stem cells in donors for hematopoietic stem cell transplantation (25).

In addition to cellular redeployment, the recovery phase of exercise, especially following very prolonged bouts of endurance-based exercise, is marked by striking alterations in the functional capacity of several blood leukocyte populations. Neutrophil bactericidal activity is greatly influenced by the intensity and duration of exercise. For example, after 1 h of cycling at 50% and 80% of $\dot{V}O_{2max}$ increases and reduces neutrophil oxidative burst activity, respectively (94). During the early stages of recovery after exercise, neutrophil bactericidal activity continues to increase after 40 min to 1 h of moderate intensity exercise, whereas it remains impaired after exhaustive or prolonged exercise (86). NK cell cytotoxicity after exercise bouts of relatively short duration tends to remain unchanged on a per cell basis during recovery (81) but may decline after very prolonged bouts (33). T-cell proliferation in response to mitogen stimulation typically decreases both during and after exercise, regardless of exercise modality, intensity, or duration (126). Prolonged exercise may also reduce T-cell homing and migration (8), LPS-induced cytokine secretion by monocytes (113), and the

percentage of T cells producing effector cytokines in response to mitogen stimulation (115). Thus, the general trend during exercise recovery is that short bouts of moderate-intensity exercise have little effect (or might even enhance) cellular immune function, whereas prolonged bouts (>1.5 h) of heavy exertion appear to reduce the normal functioning of all major immune cell subtypes. These effects may leave athletes susceptible to illness during recovery from competition or heavy training (87).

REPEATED EXERCISE BOUTS AND EXTENDED PERIODS OF INTENSE TRAINING

The repeated bout paradigm (87) proposes that, compared with a single bout of exercise, repeated exercise bouts on the same day (27, 73, 96, 102) or over several days (43) cause different changes in circulating cell counts, lymphocyte proliferation, and NK cytotoxicity. Subsequent research has investigated changes in other immune responses to repeated exercise bouts on the same day with short versus long recovery and intensified training over weeks or months. Table 1 summarizes the evidence for changes in the immune system after repeated exercise bouts and days to months of intense training.

One Versus Two Bouts of Exercise per Day

Studies on the effects of one versus two bouts of exercise on a single day have included physically active (56-58, 63, 96, 102), highly trained (23, 88), or elite (12, 73, 98, 99) participants. The exercises involved cycling (12, 27, 56-58, 63, 96, 98, 99, 102), running (23, 88), or rowing (73). The duration and intensity ranged from <15 min at maximal intensity (27, 73) up to 2 h at medium–high intensity (i.e., 60–75% $\dot{V}O_{2max}$) (56, 96). The recovery period between exercise bouts was most commonly 3–4 h but ranged from 45 min (102) to 12 h (88).

Compared with the initial bout of exercise, typically either show a greater relative change or a higher absolute value after a second bout of exercise for the following immune variables: total leukocyte count (57, 63, 99, 102), neutrophil count (23, 73, 96, 99, 102), oxidative burst (per neutrophil) (12), elastase release (per neutrophil) (57), $CD4^+$ T-cell count (73, 99, 102), whole-blood IL-8 production (23), and NK cell activation (represented by CD69 expression) (99). Conversely, lymphocyte proliferation (96, 102) and whole-blood IL-6 production (23) are typically lower following a second bout compared with the first bout of exercise.

Short Versus Long Recovery

Three studies on the effects of recovery duration between two bouts of exercise included highly trained or elite athletes who cycled for 65 min at 75% $\dot{V}O_{2max}$, twice each day, with either 3 or 6 h between the exercise bouts (12, 97, 98). A short recovery period (i.e., 3 h) induces either a greater relative increase or higher absolute values for neutrophil count (12, 97), oxidative burst activity per neutrophil (12), and $CD8^+$ T-cell and NK-cell counts (97) after the second bout of exercise. Exercise-induced changes in lymphocyte (97), monocyte, and eosinophil (12) counts; absolute oxidative burst activity (12); NK cytotoxicity (97); and plasma concentrations of IL-6 and IL-1ra (98) do not differ after a short versus long recovery period.

Consecutive or Multiple Days of Exercise

Studies detailing how the immune system responds to exercise repeated on consecutive days or every second day have included untrained or physically active participants (43, 116, 118) or well-trained or elite athletes (60, 73, 77, 79). Exercise included 3 $\times$ 6 min maximal rowing, repeated twice over 2 d (73), 1–3 h cycling (77, 79, 118) or running (60) at 50–70% $\dot{V}O_{2max}$ repeated over 3 d, every second day for 3 d (43), or daily for 7 d (116). Exercise-induced changes in plasma cytokine and elastase concentrations and cytokine mRNA expression in

leukocytes and muscle diminish over consecutive days (77, 118). By contrast, changes in total leukocyte, neutrophil, and monocyte counts (73, 116, 118), lymphocyte proliferation (79), neutrophil chemotaxis (116), leukocyte IL-1ra mRNA expression (77), plasma myeloperoxidase concentration, and salivary IgA secretion rate (79) do not change over time. Changes in lymphocyte subsets (43, 73), oxidative burst activity (79, 116, 118), salivary IgA secretion rate (60, 77), and NK-cell count and cytotoxicity (73, 79) over consecutive days are more variable.

Immune Changes Associated with Overreaching and Overtraining

Studies on short periods (2–4 weeks) of functional overreaching have reported decreases in resting neutrophil degranulation (95), lymphocyte proliferation, and antibody production (124). Neutrophil count, plasma cytokine concentrations, CD4:CD8 T-cell ratio, and salivary IgA concentration are more variable (or do not change) in response to functional overreaching (39, 95, 124). Athletes who exhibit signs of nonfunctional overreaching and/or frequent upper respiratory illness present with lower salivary IgA concentration (26, 35, 60); lower cytokine production by monocytes, neutrophils and dendritic cells (67); and a greater number of activated ($CD25^+$) lymphocytes (29). Changes in differential blood cell counts, lymphocyte subsets, and NK-cell count following extended periods of intensified training are variable (29, 35, 61). Studies of athletes exhibiting the hallmarks of overtraining syndrome—including illness—have not revealed any consistent or characteristic immune profile (30, 101).

SLEEP DISTURBANCE AND IMMUNE FUNCTION

Sleep disturbances influence immunity via activation of the hypothalamic–pituitary–adrenal axis and the sympathetic nervous-system (46). Chronic sleep disturbance and disruption to the normal circadian rhythm are associated with inflammation and desynchronization of rhythmic immune variables. These responses likely contribute to increased risk of infection, cardiovascular disease, and cancer in shift workers (21, 68). Despite evidence that athletes experience poor sleep patterns compared with nonathletes (16, 55), surprisingly little is known about how sleep disturbance influences the immune responses to exercise. Compared with normal sleep, a disrupted night's sleep appears to prime the immune system and enhance immunosurveillance by stimulating total lymphocytes, $CD8^+$ T cells, NK cells, and $\gamma\delta$ T cells to leave the blood and migrate to potential sites of infection during the early recovery period after exercise (45). By contrast, other studies indicate that a night without sleep does not influence leukocyte trafficking, neutrophil degranulation, or mucosal immunity at rest or after exercise (31, 93). Subtle immune changes have been observed after a night without sleep, including a shift toward a T helper 2 cytokine profile (46).

It is uncertain whether these subtle immune modifications with acute sleep loss are clinically meaningful. When considering the potential effects of poor sleep on immunity in athletes, it is important to distinguish between acute and chronic sleep disturbance. Chronic sleep disturbance (12 nights, 50% sleep loss) increases the plasma inflammation markers C-reactive protein and IL-6 (38). However, intervening daytime naps can counter this apparent inflammatory response (103). Short sleep duration (<7 h/night for 7 d) decreases the response to hepatitis B vaccination and the likelihood of clinical protection (90). Similarly, a night of

wakefulness after hepatitis A vaccination decreases the specific antibody response 2–4 months later (52). People who experience poor quality sleep and/or regular sleep deprivation also have a 4–5-times greater risk of developing the common cold (16, 91). Continued research efforts should be directed toward monitoring and improving sleep in athletes and understanding the implications for immune health.

NUTRITIONAL INTERVENTIONS FOR RESTORING IMMUNE FUNCTION AFTER EXERCISE

Research over the last 30 years has investigated whether nutritional strategies counteract exercise-induced immunodepression and systemic inflammation (32, 125). A comprehensive review of the literature on these is beyond the scope of this mini-review; other more detailed reviews on this topic are available (e.g., (32, 125). Here, we focus on the most effective nutritional strategies—primarily carbohydrate ingestion—for restoring systemic immune function in the first few hours after exercise (9, 10, 19, 51, 65, 76, 78, 82, 83) and over consecutive days (7). We also assess whether the timing of nutritional interventions (i.e., before, during or after exercise) influence their effectiveness (50, 59).

Carbohydrate Supplementation before and/or during Exercise

Carbohydrate supplementation during prolonged, intense exercise consistently attenuates exercise-induced increases in circulating cytokines (74, 125), and the re-distribution of neutrophils (74, 76, 78), monocytes (76, 82), natural killer cells (78), and lymphocytes (51). The immunomodulatory effects of carbohydrates arise from better maintenance of blood glucose concentrations and blunted release of stress hormones such as catecholamines and glucocorticoids during and after exercise (51, 76, 78, 82, 83). Although the systemic release

of IL-6 during exercise is related to muscle glycogen depletion (114), the precise mechanism by which carbohydrate supplementation reduces systemic IL-6 release from contracting muscle during exercise is not clear, because carbohydrate supplementation does not alter muscle glycogen content (75).

In several studies, the immunomodulatory effects of carbohydrate supplementation were observed to 'carry over' into the recovery period (i.e., ≥ 2 h post-exercise) (51, 76, 78, 82, 83). Nieman et al reported that carbohydrate supplementation during 2.5 h high-intensity running reduces the number of neutrophils (immediately and 1.5 h post-exercise), monocytes (immediately and 6 h post-exercise) and lymphocytes (immediately and 3 h post-exercise) (76, 78). Extending carbohydrate ingestion to the post-exercise recovery period also reduces neutrophil count (74), blood granulocyte and monocyte phagocytosis 6 h post-exercise (82). Lancaster et al showed that carbohydrate consumption (30 and 60 g per hour) during 2.5 h cycling minimized the suppression of $CD4^+$ and $CD8^+$ T lymphocyte that express and produce IFN-γ during the 2 h following exercise (51).

Considering that exercise-induced responses of the adaptive immune system are relatively slow (125), it is important to assess whether these effects are maintained over consecutive days of exercise. Carbohydrate ingestion before, during and after two exercise bouts on two consecutive days attenuated the decrease in antigen-stimulated proliferative lymphocyte responses before exercise on the second day (7). Carbohydrate ingestion also enhanced lymphocyte proliferative responses to mitogen stimulation post-exercise on the second day (7). These findings suggest that carbohydrates may help to diminish potential cumulative immunodepression over consecutive days of exercise.

The immunomodulatory effects of carbohydrate may depend on the timing of

carbohydrate intake. The ingestion of a glucose solution 15 min—but not 75 min—before 1 h high-intensity cycling prevented immunoendocrine perturbations (50). The lack of an effect of carbohydrates ingested 75 min pre-exercise was potentially associated with an insulin-induced decrease in the plasma glucose concentration prior to exercise, which in turn might have enhanced immunoendocrine responses (50). Carbohydrate ingestion during either the first or the second of two 90-min bouts of cycling on the same day better maintained plasma glucose and attenuated plasma stress hormone responses to the second bout (59). By contrast, carbohydrate ingestion during the 2 h recovery period between these exercise bouts had no such effects (59). These findings suggest beneficial effects of a timely carbohydrate supplementation (i.e., shortly before and/or during exercise) on immune responses to exercise. This may be particularly relevant with more prolonged and/or intense exercise protocols, and when the recovery duration between two consecutive exercise bouts is short.

Carbohydrate ingestion does not influence all aspects of the immune system. For example, carbohydrate supplementation does not alter the exercise-induced suppression of natural killer cell function (78) or salivary IgA secretion (18). Importantly, it remains unclear whether the immunomodulatory effects of carbohydrates have clinical relevance for resistance to illness or adaptation of the immune system to regular exercise stress (32, 125). Recent evidence indicates that carbohydrate supplementation during prolonged exercise blunts exercise-induced immune-endocrine perturbations, but does not prevent the suppression of in vivo immunity (22). More research is required to examine the effects of carbohydrates (or other nutritional strategies) on in vivo immune function in response to acute and chronic exercise.

Dietary Carbohydrate Intake after Glycogen-Depletion

Some studies have investigated the effects of dietary carbohydrate intake on immune responses to consecutive days of exercise intended to deplete muscle glycogen (9, 10, 34, 65). A higher carbohydrate intake consistently attenuated certain components of immunodepression well into the recovery period (i.e., ≥2 hours post-exercise) after the second exercise session (10, 34, 65). Athletic training often involves conditions of low carbohydrate availability e.g., due to abbreviated recovery periods and/or as part of 'train low-compete high' training regime (41, 42). These investigations therefore have particular practical implications. Compared with a higher carbohydrate intake (8 g/kg/d), very low carbohydrate intake (0.5 g/kg/d) leads to greater perturbation in leukocyte subsets during recovery from exercise (65). These effects may be related to sustained elevation of plasma cortisol concentration (65). Bishop et al observed that compared with a low-carbohydrate diet (1.1 g/kg/d), a high- (8.4 g/kg/d) for 3 d after glycogen-lowering cycling attenuated plasma cortisol and cytokine concentrations, circulating total leukocyte and neutrophil counts following subsequent exercise (10).

Consuming a high carbohydrate diet (8.5 g/kg/d) also reduces overreaching symptoms during 11 d of intense training, compared with moderate carbohydrate consumption (5.5 g/kg/d) (1). The periodic implementation of 'train-low' strategies (e.g., by commencing training with low muscle glycogen stores) may further amplify metabolic adaptations in skeletal muscle (41, 42). When considering their dietary carbohydrate intake, athletes should aim to achieve a balance between minimizing immunodepression and maximizing metabolic adaptations in skeletal muscle. In view of the detrimental effects of low carbohydrate availability on the immune system, *chronic* carbohydrate restriction should be avoided during intense periods of training (32, 41). Additional research is warranted to better understand the effect of long-term training consisting of intermittent 'train-low' sessions on immune function

and susceptibility to illness.

Dietary Protein Intake and Post-Exercise Protein Supplementation

Recognizing the importance of protein for immunocompetence (15), there are benefits of post-exercise protein ingestion (18, 19, 69) or a diet high in protein (128) on immune responses to exercise. Based on previous results indicating that the exercise-induced lymphocyte trafficking was impaired during high-intensity training, Witard et al examined whether a high protein diet can restore these impaired immune responses (128). Consuming a high protein diet (3 g/kg/d) helped to minimize exercise-induced changes in lymphocyte distribution during a period of intense training (128). Interestingly, an energy- and carbohydrate-matched normal protein diet (1.5 g/kg/d) failed to provide the same benefit (128). The high-protein diet was also associated with fewer self-reported upper-respiratory illnesses (128). Another study demonstrated that protein and leucine supplementation for 1–3 h post-exercise during 6 d of high-intensity training enhanced neutrophil respiratory burst activity after the last exercise session (69). Consuming a carbohydrate-protein solution immediately—but not 1 h—after exercise prevents a decrease in neutrophil degranulation during the post-exercise recovery period (19).

Recent research has shown that the timing, distribution and amount of post-exercise protein intake modulate the blood and tissue availability of protein/amino acids and adaptive responses of skeletal muscle (3, 42). Notably, amino acid-sensitive mammalian target of rapamycin (mTOR) signaling is also a key mechanism underlying leukocyte trafficking (110). More studies are therefore needed to examine whether different post-exercise protein feeding patterns influence immune function during recovery from exercise.

Antioxidants and Phytochemicals

Except for carbohydrate supplementation, evidence for effective nutritional countermeasures to exercise-induced immune alterations is limited (32, 125). Among other types of nutritional supplements (e.g., probiotics and vitamin D (for reviews see (32, 125)), antioxidants and phytochemicals such as quercetin have been studied for their potential capacity to minimize immune perturbations—particularly during exercise recovery (75, 77, 79, 80). Some data point toward beneficial effects of quercetin supplementation on immune health after intense exercise (77, 79). Other findings suggest an increased need for nutritional antioxidants during the first 24 h of recovery from intense exercise lasting several hours such as an Ironman triathlon (71). However, taken together, the present literature is not sufficiently robust to recommend supplementation with phytochemicals or antioxidants to prevent immune suppression and illness in athletes and exercising individuals. Athletes often take high doses of antioxidant/phytochemical supplements in the belief that this will reduce their risk of illness (47). However, high doses of antioxidant/phytochemical supplements can interfere with training adaptations (42, 72). A natural diet rich in fruits, vegetables, whole grains and nuts delivers antioxidants and phytochemicals in physiologically effective amounts that are most likely sufficient to help maintain immune function following exercise and during exercise training (32, 72, 125).

OTHER STRATEGIES FOR RESTORING IMMUNE FUNCTION AFTER EXERCISE

In addition to nutritional interventions, other research has examined the efficacy of nonsteroidal anti-inflammatory drugs (NSAIDs) and various physical therapies for restoring immune function after exercise. Some studies (13, 20, 70, 89, 120, 127) have shown effects of NSAIDs, but other human studies have failed to demonstrate any effects of NSAIDs (122,

123), cryotherapy (44), compression garments (5, 37, 92), active recovery (2, 119), or other physical therapies (28) on immune responses during recovery from exercise (Fig. 2). Despite this lack of empirical evidence for the benefits of NSAIDs and physical therapies for restoring immune function after exercise, some of these treatments are associated with positive psychological outcomes and other effects not related to immune function after exercise (4, 17). Therefore, although the physiological effects of these physical treatments are not understood fully at the present time, they may confer some important benefits for athletes, which may involve the immune system—perhaps indirectly.

THEORETICAL AND PRACTICAL CONSIDERATIONS

The redeployment of effector lymphocytes from blood to peripheral tissues is seen as an integral part of the physiological stress response and the immune system's response to prepare the body for potential injury by mobilizing its 'troops' (cells) to increase immunosurveillance (24). Therefore, immune integrity during exercise recovery may be characterized by the host's ability to redeploy effector lymphocytes effectively to the peripheral tissues. Lymphocyte redeployment may be impaired following very prolonged bouts of exercise or in athletes who are overreaching. The redeployment of $CD8^+$ T cells both during and after exercise is significantly reduced after 1 wk of high-intensity training compared with normal training (129). Specifically, the egress of $CD8^+$ T cells was 1.4-fold higher after normal compared with high-intensity training.

Considering other evidence that stress-induced leukocyte redeployment is linked to poor clinical outcomes following surgery (100), immune cell redistribution and infection risk after

exercise warrants further investigation. High-volume exercise training may impair the redeployment of viral-specific T cells and NK cells, thereby reducing antiviral 'patrolling' during exercise recovery (Fig. 1). Herpes viruses such as EBV and CMV are highly prevalent in the population, and reactivation of these viruses from a latent state is indicative of systemic immunodepression (107). EBV viral DNA is present in saliva from athletes after even short periods of high-intensity training (36), but it is unknown whether this reflects training-induced impairment in the trafficking of virus-fighting lymphocytes.

Cellular immune function in response to exercise is typically assessed in isolated cells ex vivo, or at the cell population level in the blood compartment. This approach can make it difficult to interpret changes in immune cell function after exercise because of the massive alterations in the cellular composition of discrete leukocyte subtypes. On the one hand, it seems intuitive to interpret lower immune cell function measured in blood during the early stages of exercise recovery as indicative of immunodepression. On the other hand, it is equally possible that, after exercise, the most functional immune cells (i.e., those with effector phenotypes and high tissue-migrating potential) are redeployed to other areas of the body where they are needed. If true, this suggests that systemic immunosurveillance may be enhanced during exercise recovery, despite an apparent depressed profile in the blood compartment.

It is difficult to determine the biological significance of exercise-induced changes in immune cell function when assessed *in vitro*. This is because cell function is typically assessed relative to the total cell population (e.g., percentage of T cells responding to mitogen stimulation, number of target cells killed per NK-cell, oxidative burst activity per neutrophil, etc.) without accounting for exercise-induced changes in the subset composition of these cell

populations. For example, the proportion of NK cells expressing the activating receptor, NKG2C, is markedly elevated during exercise recovery (6). As a result, NK-cell cytotoxic activity for the total NK-cell population increases markedly when an NKG2C-sensitive target cell is used to assess NK-cell function (6). Conversely, when an NKG2C-insensitive target cell line (K562) is used, NK-cell killing is not affected by exercise (6). Therefore, the proportional shifts in the composition of cell subtypes should be considered when interpreting exercise-induced changes in immune cell function at the total cell population level in vitro. Moreover, assessment of in vitro immune cell function using venous blood samples does not account for the complex interactions among immune cells and soluble factors within tissues (e.g., gut, lungs, skin). However, some evidence suggests that reduced immune cell function in vitro may coincide with changes in vivo and rates of illness (14, 40).

The validity of the original paradigm of cumulative immunodepression with repeated bouts of exercise (87) is somewhat difficult to assess. Months of intense training increase the incidence of illness in elite athletes (26, 30, 35). However, based on these studies, we can only assume—but not assert—that increased incidence of illness results from an imbalance between training and recovery. Research that has systematically manipulated the balance between training and recovery has not identified any immune variables that are consistently depressed as a result of insufficient recovery after exercise. However, with one exception (79), these studies have not tracked the incidence of illness after repeated bouts of exercise.

Reduced salivary IgA concentration and secretion rate (amount of IgA secreted over a fixed period) may predispose athletes to illness in the long term (26, 35). IgA binds micro-organisms such as bacteria and viruses in the mucosa so that they can be destroyed by immune cells. However, short-term changes in salivary IgA concentration and secretion rate

after repeated bouts of exercise are variable (56, 58, 60, 79). Salivary IgA concentration and secretion rate may decrease incrementally over longer periods. The repeated exercise models used in many of the studies described above induce only acute fatigue (64). Accordingly, smaller exercise-induced changes in immune variables following repeated bouts of exercise may actually represent positive adaptation of the immune system—as opposed to depression of immunity that may lead to illness.

Pedersen et al (87) suggested that there is a critical threshold for exercise intensity and duration that determines the risk of immunodepression after repeated bouts of exercise. However, no studies have systematically determined the effects of repeated exercise bouts of different intensity and duration. There are also no data on the effects of repeated bouts of anaerobic or resistance/strength exercise, or a combination of different types of exercise on the same day. The large gaps in Table 1 show that much remains to be learned about the effects of repeated bouts of exercise on the immune system.

Among various nutritional interventions that have been studied to counteract immunodepression during exercise recovery, carbohydrate supplementation has proven the most effective. A balanced and well-diversified diet that meets the energy demands in athletes and exercising individuals is certainly a key component to maintain immune function in response to strenuous exercise and intense periods of training. Additional research is warranted to investigate how the timing and pattern in the ingestion of nutrients—particularly carbohydrates and protein/amino acids—influence recovery of the immune system after exercise.

Sleep disturbances can depress immunity, increase inflammation, and promote adverse health outcomes in the general population. However, the limited data available on how sleep

455 disturbances influence immune responses to exercise are inconsistent. Physical treatments
456 that are used after exercise (e.g., hydrotherapy and massage) may enhance the athlete's
457 sense of well-being, and should be considered as adjunct therapies for maintaining immune
458 health.

459 **ACKNOWLEDGMENTS**

460 Jonathan Peake is supported by funding from the Centre of Excellence for Applied Sport
461 Science Research at the Queensland Academy of Sport, Brisbane, Australia. We acknowledge
462 copyediting by Laurel T Mackinnon, PhD, FACSM, ELS.

REFERENCES

1. **Achten J, Halson SL, Moseley L, Rayson MP, Casey A, and Jeukendrup AE**. Higher dietary carbohydrate content during intensified running training results in better maintenance of performance and mood state. *J Appl Physiol (1985)* 96: 1331-1340, 2004.
2. **Andersson H, Bohn SK, Raastad T, Paulsen G, Blomhoff R, and Kadi F**. Differences in the inflammatory plasma cytokine response following two elite female soccer games separated by a 72-h recovery. *Scand J Med Sci Sports* 20: 740-747, 2010.
3. **Areta JL, Burke LM, Ross ML, Camera DM, West DW, Broad EM, Jeacocke NA, Moore DR, Stellingwerff T, Phillips SM, Hawley JA, and Coffey VG**. Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis. *J Physiol* 591: 2319-2331, 2013.
4. **Arroyo-Morales M, Olea N, Ruiz C, del Castilo Jde D, Martinez M, Lorenzo C, and Diaz-Rodriguez L**. Massage after exercise responses of immunologic and endocrine markers: a randomized single-blind placebo-controlled study. *J Strength Cond Res* 23: 638-644, 2009.
5. **Bieuzen F, Brisswalter J, Easthope C, Vercruyssen F, Bernard T, and Hausswirth C**. Effect of wearing compression stockings on recovery after mild exercise-induced muscle damage. *Int J Sports Physiol Perform* 9: 256-264, 2014.
6. **Bigley AB, Rezvani K, Chew C, Sekine T, Pistillo M, Crucian B, Bollard CM, and Simpson RJ**. Acute exercise preferentially redeploys NK-cells with a highly-differentiated phenotype and augments cytotoxicity against lymphoma and multiple myeloma target cells. *Brain Behav Immun* 39: 160-171, 2014.
7. **Bishop NC, Walker GJ, Bowley LA, Evans KF, Molyneux K, Wallace FA, and Smith AC**. Lymphocyte responses to influenza and tetanus toxoid in vitro following intensive exercise and carbohydrate ingestion on consecutive days. *J Appl Physiol (1985)* 99: 1327-1335, 2005.
8. **Bishop NC, Walker GJ, Gleeson M, Wallace FA, and Hewitt CR**. Human T lymphocyte migration towards the supernatants of human rhinovirus infected airway epithelial cells: influence of exercise and carbohydrate intake. *Exerc Immunol Rev* 15: 127-144, 2009.
9. **Bishop NC, Walsh NP, Haines DL, Richards EE, and Gleeson M**. Pre-exercise carbohydrate status and immune responses to prolonged cycling: I. Effect on neutrophil degranulation. *Int J Sport Nutr Exerc Metab* 11: 490-502, 2001.
10. **Bishop NC, Walsh NP, Haines DL, Richards EE, and Gleeson M**. Pre-exercise carbohydrate status and immune responses to prolonged cycling: II. Effect on plasma cytokine concentration. *Int J Sport Nutr Exerc Metab* 11: 503-512, 2001.
11. **Booth S, Florida-James GD, McFarlin BK, Spielmann G, O'Connor DP, and Simpson RJ**. The impact of acute strenuous exercise on TLR2, TLR4 and HLA.DR expression on human blood monocytes induced by autologous serum. *Eur J Appl Physiol* 110: 1259-1268, 2010.
12. **Boyum A, Ronsen O, Tennfjord VA, Tollefsen S, Haugen AH, Opstad PK, and Bahr R**. Chemiluminescence response of granulocytes from elite athletes during recovery from one or two intense bouts of exercise. *Eur J Appl Physiol* 88: 20-28, 2002.
13. **Broadbent S, Rousseau JJ, Thorp RM, Choate SL, Jackson FS, and Rowlands DS**. Vibration therapy reduces plasma IL6 and muscle soreness after downhill running. *Br J Sports Med* 44: 888-894, 2010.
14. **Bruunsgaard H, Hartkopp A, Mohr T, Konradsen H, Heron I, Mordhorst CH, and Pedersen BK**. In vivo cell-mediated immunity and vaccination response following prolonged, intense exercise. *Med Sci Sports Exerc* 29: 1176-1181, 1997.
15. **Calder PC, and Jackson AA**. Undernutrition, infection and immune function. *Nutr Res Rev* 13: 3-29, 2000.
16. **Cohen S, Doyle WJ, Alper CM, Janicki-Deverts D, and Turner RB**. Sleep habits and susceptibility to the common cold. *Arch Intern Med* 169: 62-67, 2009.

17. **Cortis C, Tessitore A, D'Artibale E, Meeusen R, and Capranica L**. Effects of post-exercise recovery interventions on physiological, psychological, and performance parameters. *Int J Sports Med* 31: 327-335, 2010.
18. **Costa RJ, Fortes MB, Richardson K, Bilzon JL, and Walsh NP**. The effects of postexercise feeding on saliva antimicrobial proteins. *Int J Sport Nutr Exerc Metab* 22: 184-191, 2012.
19. **Costa RJ, Oliver SJ, Laing SJ, Waiters R, Bilzon JL, and Walsh NP**. Influence of timing of postexercise carbohydrate-protein ingestion on selected immune indices. *Int J Sport Nutr Exerc Metab* 19: 366-384, 2009.
20. **Crane JD, Ogborn DI, Cupido C, Melov S, Hubbard A, Bourgeois JM, and Tarnopolsky MA**. Massage therapy attenuates inflammatory signaling after exercise-induced muscle damage. *Sci Transl Med* 4: 119ra113, 2012.
21. **Cuesta M, Boudreau P, Dubeau-Laramee G, Cermakian N, and Boivin DB**. Simulated night shift disrupts circadian rhythms of immune functions in humans. *J Immunol* 196: 2466-2475, 2016.
22. **Davison G, Kehaya C, Diment BC, and Walsh NP**. Carbohydrate supplementation does not blunt the prolonged exercise-induced reduction of in vivo immunity. *Eur J Nutr* 55: 1583-1593, 2016.
23. **Degerstrom J, and Osterud B**. Increased inflammatory response of blood cells to repeated bout of endurance exercise. *Med Sci Sports Exerc* 38: 1297-1303, 2006.
24. **Dhabhar FS**. Enhancing versus Suppressive Effects of Stress on Immune Function: Implications for Immunoprotection versus Immunopathology. *Allergy Asthma Clin Immunol* 4: 2-11, 2008.
25. **Emmons R, Niemiro GM, Owolabi O, and De Lisio M**. Acute exercise mobilizes hematopoietic stem and progenitor cells and alters the mesenchymal stromal cell secretome. *J Appl Physiol* 120: 624-632, 2016.
26. **Fahlman MM, and Engels HJ**. Mucosal IgA and URTI in American college football players: a year longitudinal study. *Med Sci Sports Exerc* 37: 374-380, 2005.
27. **Field CJ, Gougeon R, and Marliss EB**. Circulating mononuclear cell numbers and function during intense exercise and recovery. *J Appl Physiol* 71: 1089-1097, 1991.
28. **Finberg M, Braham R, Goodman C, Gregory P, and Peeling P**. Effects of electrostimulation therapy on recovery from acute team-sport activity. *Int J Sports Physiol Perform* 8: 293-299, 2013.
29. **Fry RW, Grove JR, Morton AR, Zeroni PM, Gaudieri S, and Keast D**. Psychological and immunological correlates of acute overtraining. *Br J Sports Med* 28: 241-246, 1994.
30. **Gabriel HH, Urhausen A, Valet G, Heidelbach U, and Kindermann W**. Overtraining and immune system: a prospective longitudinal study in endurance athletes. *Med Sci Sports Exerc* 30: 1151-1157, 1998.
31. **Gillum TL, Kuennen MR, Castillo MN, Williams NL, and Jordan-Patterson AT**. Exercise, but not acute sleep loss, increases salivary antimicrobial protein secretion. *J Strength Cond Res* 29: 1359-1366, 2015.
32. **Gleeson M**. Immunological aspects of sport nutrition. *Immunol Cell Biol* 94: 117-123, 2016.
33. **Gleeson M, and Bishop NC**. The T cell and NK cell immune response to exercise. *Ann Transplant* 10: 43-48, 2005.
34. **Gleeson M, Blannin AK, Walsh NP, Bishop NC, and Clark AM**. Effect of low- and high-carbohydrate diets on the plasma glutamine and circulating leukocyte responses to exercise. *Int J Sport Nutr* 8: 49-59, 1998.
35. **Gleeson M, McDonald WA, Cripps AW, Pyne DB, Clancy RL, and Fricker PA**. The effect on immunity of long-term intensive training in elite swimmers. *Clin Exp Immunol* 102: 210-216, 1995.
36. **Gleeson M, Pyne DB, Austin JP, Lynn Francis J, Clancy RL, McDonald WA, and Fricker PA**. Epstein-Barr virus reactivation and upper-respiratory illness in elite swimmers. *Med Sci Sports Exerc* 34: 411-417, 2002.
37. **Goto K, and Morishima T**. Compression garment promotes muscular strength recovery after resistance exercise. *Med Sci Sports Exerc* 46: 2265-2270, 2014.

38. **Haack M, Sanchez E, and Mullington JM**. Elevated inflammatory markers in response to prolonged sleep restriction are associated with increased pain experience in healthy volunteers. *Sleep* 30: 1145-1152, 2007.

39. **Halson SL, Lancaster GI, Jeukendrup AE, and Gleeson M**. Immunological responses to overreaching in cyclists. *Med Sci Sports Exerc* 35: 854-861, 2003.

40. **Harper Smith AD, Coakley SL, Ward MD, Macfarlane AW, Friedmann PS, and Walsh NP**. Exercise-induced stress inhibits both the induction and elicitation phases of in vivo T-cell-mediated immune responses in humans. *Brain Behav Immun* 25: 1136-1142, 2011.

41. **Hawley JA, and Burke LM**. Carbohydrate availability and training adaptation: effects on cell metabolism. *Exerc Sport Sci Rev* 38: 152-160, 2010.

42. **Hawley JA, Burke LM, Phillips SM, and Spriet LL**. Nutritional modulation of training-induced skeletal muscle adaptations. *J Appl Physiol (1985)* 110: 834-845, 2011.

43. **Hoffman-Goetz L, Simpson JR, Cipp N, Arumugam Y, and Houston ME**. Lymphocyte subset responses to repeated submaximal exercise in men. *J Appl Physiol* 68: 1069-1074, 1990.

44. **Hohenauer E, Taeymans J, Baeyens JP, Clarys P, and Clijsen R**. The effect of post-exercise cryotherapy on recovery characteristics: a systematic review and meta-analysis. *PLoS One* 10: e0139028, 2015.

45. **Ingram LA, Simpson RJ, Malone E, and Florida-James GD**. Sleep disruption and its effect on lymphocyte redeployment following an acute bout of exercise. *Brain Behav Immun* 47: 100-108, 2015.

46. **Irwin MR**. Why sleep is important for health: a psychoneuroimmunology perspective. *Annu Rev Psychol* 66: 143-172, 2015.

47. **Knez WL, and Peake JM**. The prevalence of vitamin supplementation in ultraendurance triathletes. *Int J Sport Nutr Exerc Metab* 20: 507-514, 2010.

48. **Kruger K, Lechtermann A, Fobker M, Volker K, and Mooren FC**. Exercise-induced redistribution of T lymphocytes is regulated by adrenergic mechanisms. *Brain Behav Immun* 22: 324-338, 2008.

49. **Kruger K, Pilat C, Schild M, Lindner N, Frech T, Muders K, and Mooren FC**. Progenitor cell mobilization after exercise is related to systemic levels of G-CSF and muscle damage. *Scand J Med Sci Sports* 25: e283-291, 2015.

50. **Lancaster GI, Jentjens RL, Moseley L, Jeukendrup AE, and Gleeson M**. Effect of pre-exercise carbohydrate ingestion on plasma cytokine, stress hormone, and neutrophil degranulation responses to continuous, high-intensity exercise. *Int J Sport Nutr Exerc Metab* 13: 436-453, 2003.

51. **Lancaster GI, Khan Q, Drysdale PT, Wallace F, Jeukendrup AE, Drayson MT, and Gleeson M**. Effect of prolonged exercise and carbohydrate ingestion on type 1 and type 2 T lymphocyte distribution and intracellular cytokine production in humans. *J Appl Physiol (1985)* 98: 565-571, 2005.

52. **Lange T, Dimitrov S, Bollinger T, Diekelmann S, and Born J**. Sleep after vaccination boosts immunological memory. *J Immunol* 187: 283-290, 2011.

53. **Lansford KA, Shill DD, Dicks AB, Marshburn MP, Southern WM, and Jenkins NT**. Effect of acute exercise on circulating angiogenic cell and microparticle populations. *Exp Physiol* 101: 155-167, 2016.

54. **LaVoy EC, Bosch JA, Lowder TW, and Simpson RJ**. Acute aerobic exercise in humans increases cytokine expression in CD27(-) but not CD27(+) CD8(+) T-cells. *Brain Behav Immun* 27: 54-62, 2013.

55. **Leeder J, Glaister M, Pizzoferro K, Dawson J, and Pedlar C**. Sleep duration and quality in elite athletes measured using wristwatch actigraphy. *J Sports Sci* 30: 541-545, 2012.

56. **Li TL, and Gleeson M**. The effect of single and repeated bouts of prolonged cycling and circadian variation on saliva flow rate, immunoglobulin A and alpha-amylase responses. *J Sports Sci* 22: 1015-1024, 2004.

57. **Li TL, and Gleeson M**. The effect of single and repeated bouts of prolonged cycling on leukocyte redistribution, neutrophil degranulation, IL-6, and plasma stress hormone responses. *Int J Sport Nutr Exerc Metab* 14: 501-516, 2004.

58. **Li TL, and Gleeson M**. The effects of carbohydrate supplementation during repeated bouts of prolonged exercise on saliva flow rate and immunoglobulin A. *J Sports Sci* 23: 713-722, 2005.

59. **Li TL, and Gleeson M**. The effects of carbohydrate supplementation during the second of two prolonged cycling bouts on immunoendocrine responses. *Eur J Appl Physiol* 95: 391-399, 2005.
60. **Mackinnon LT, and Hooper S**. Mucosal (secretory) immune system responses to exercise of varying intensity and during overtraining. *Int J Sports Med* 15 Suppl 3: S179-183, 1994.
61. **Mackinnon LT, Hooper SL, Jones S, Gordon RD, and Bachmann AW**. Hormonal, immunological, and hematological responses to intensified training in elite swimmers. *Med Sci Sports Exerc* 29: 1637-1645, 1997.
62. **Mars M, Govender S, Weston A, Naicker V, and Chuturgoon A**. High intensity exercise: a cause of lymphocyte apoptosis? *Biochem Biophys Res Commun* 249: 366-370, 1998.
63. **McFarlin B, Mitchell JB, McFarlin MA, and Steinhoff GM**. Repeated endurance exercise affects leukocyte number but not NK cell activity. *Med Sci Sports Exerc* 35: 1130-1138, 2003.
64. **Meeusen R, Duclos M, Foster C, Fry A, Gleeson M, Nieman D, Raglin J, Rietjens G, Steinacker J, and Urhausen A**. Prevention, diagnosis, and treatment of the overtraining syndrome: joint consensus statement of the European College of Sport Science and the American College of Sports Medicine. *Med Sci Sports Exerc* 45: 186-205, 2013.
65. **Mitchell JB, Pizza FX, Paquet A, Davis BJ, Forrest MB, and Braun WA**. Influence of carbohydrate status on immune responses before and after endurance exercise. *J Appl Physiol (1985)* 84: 1917-1925, 1998.
66. **Mooren FC, Bloming D, Lechtermann A, Lerch MM, and Volker K**. Lymphocyte apoptosis after exhaustive and moderate exercise. *J Appl Physiol* 93: 147-153, 2002.
67. **Morgado JM, Rama L, Silva I, de Jesus Inacio M, Henriques A, Laranjeira P, Pedreiro S, Rosado F, Alves F, Gleeson M, Pais ML, Paiva A, and Teixeira AM**. Cytokine production by monocytes, neutrophils, and dendritic cells is hampered by long-term intensive training in elite swimmers. *Eur J Appl Physiol* 112: 471-482, 2012.
68. **Mullington JM, Simpson NS, Meier-Ewert HK, and Haack M**. Sleep loss and inflammation. *Best Pract Res Clin Endocrinol Metab* 24: 775-784, 2010.
69. **Nelson AR, Jackson L, Clarke J, Stellingwerff T, Broadbent S, and Rowlands DS**. Effect of post-exercise protein-leucine feeding on neutrophil function, immunomodulatory plasma metabolites and cortisol during a 6-day block of intense cycling. *Eur J Appl Physiol* 113: 2211-2222, 2013.
70. **Nemet D, Meckel Y, Bar-Sela S, Zaldivar F, Cooper DM, and Eliakim A**. Effect of local cold-pack application on systemic anabolic and inflammatory response to sprint-interval training: a prospective comparative trial. *Eur J Appl Physiol* 107: 411-417, 2009.
71. **Neubauer O, Reichhold S, Nics L, Hoelzl C, Valentini J, Stadlmayr B, Knasmuller S, and Wagner KH**. Antioxidant responses to an acute ultra-endurance exercise: impact on DNA stability and indications for an increased need for nutritive antioxidants in the early recovery phase. *Br J Nutr* 104: 1129-1138, 2010.
72. **Neubauer O, and Yfanti C**. Antioxidants in Athlete's Basic Nutrition: Considerations towards a Guideline for the Intake of Vitamin C and Vitamin E. In: *Antioxidants in Sport Nutrition*, edited by Lamprecht M. Boca Raton (FL): 2015.
73. **Nielsen HB, Secher NH, Christensen NJ, and Pedersen BK**. Lymphocytes and NK cell activity during repeated bouts of maximal exercise. *Am J Physiol* 271: R222-227, 1996.
74. **Nieman DC**. Influence of carbohydrate on the immune response to intensive, prolonged exercise. *Exerc Immunol Rev* 4: 64-76, 1998.
75. **Nieman DC, Davis JM, Henson DA, Walberg-Rankin J, Shute M, Dumke CL, Utter AC, Vinci DM, Carson JA, Brown A, Lee WJ, McAnulty SR, and McAnulty LS**. Carbohydrate ingestion influences skeletal muscle cytokine mRNA and plasma cytokine levels after a 3-h run. *J Appl Physiol* 94: 1917-1925, 2003.
76. **Nieman DC, Fagoaga OR, Butterworth DE, Warren BJ, Utter A, Davis JM, Henson DA, and Nehlsen-Cannarella SL**. Carbohydrate supplementation affects blood granulocyte and monocyte trafficking but not function after 2.5 h or running. *Am J Clin Nutr* 66: 153-159, 1997.

77. **Nieman DC, Henson DA, Davis JM, Angela Murphy E, Jenkins DP, Gross SJ, Carmichael MD, Quindry JC, Dumke CL, Utter AC, McAnulty SR, McAnulty LS, Triplett NT, and Mayer EP**. Quercetin's influence on exercise-induced changes in plasma cytokines and muscle and leukocyte cytokine mRNA. *J Appl Physiol* 103: 1728-1735, 2007.
78. **Nieman DC, Henson DA, Garner EB, Butterworth DE, Warren BJ, Utter A, Davis JM, Fagoaga OR, and Nehlsen-Cannarella SL**. Carbohydrate affects natural killer cell redistribution but not activity after running. *Med Sci Sports Exerc* 29: 1318-1324, 1997.
79. **Nieman DC, Henson DA, Gross SJ, Jenkins DP, Davis JM, Murphy EA, Carmichael MD, Dumke CL, Utter AC, McAnulty SR, McAnulty LS, and Mayer EP**. Quercetin reduces illness but not immune perturbations after intensive exercise. *Med Sci Sports Exerc* 39: 1561-1569, 2007.
80. **Nieman DC, Henson DA, McAnulty SR, McAnulty LS, Morrow JD, Ahmed A, and Heward CB**. Vitamin E and immunity after the Kona Triathlon World Championship. *Med Sci Sports Exerc* 36: 1328-1335, 2004.
81. **Nieman DC, Miller AR, Henson DA, Warren BJ, Gusewitch G, Johnson RL, Davis JM, Butterworth DE, and Nehlsen-Cannarella SL**. Effects of high- vs moderate-intensity exercise on natural killer cell activity. *Med Sci Sports Exerc* 25: 1126-1134, 1993.
82. **Nieman DC, Nehlsen-Cannarella SL, Fagoaga OR, Henson DA, Utter A, Davis JM, Williams F, and Butterworth DE**. Effects of mode and carbohydrate on the granulocyte and monocyte response to intensive, prolonged exercise. *J Appl Physiol (1985)* 84: 1252-1259, 1998.
83. **Nieman DC, Nehlsen-Cannarella SL, Fagoaga OR, Henson DA, Utter A, Davis JM, Williams F, and Butterworth DE**. Influence of mode and carbohydrate on the cytokine response to heavy exertion. *Med Sci Sports Exerc* 30: 671-678, 1998.
84. **Peake J**. Interrelations between aute and chronic stress and the immune and endocrine systems. In: *Endocrinology of physical activity and sport*, edited by Constantini N, and Hackney A. New York: Springer, 2013, p. 258-280.
85. **Peake J, Nosaka K, and Suzuki K**. Characterization of inflammatory responses to eccentric exercise in humans. *Exerc Immunol Rev* 11: 64-85, 2005.
86. **Peake JM**. Exercise-induced alterations in neutrophil degranulation and respiratory burst activity: possible mechanisms of action. *Exerc Immunol Rev* 8: 49-100, 2002.
87. **Pedersen BK, Rohde T, and Ostrowski K**. Recovery of the immune system after exercise. *Acta Physiol Scand* 162: 325-332, 1998.
88. **Peeling P, Dawson B, Goodman C, Landers G, Wiegerinck ET, Swinkels DW, and Trinder D**. Cumulative effects of consecutive running sessions on hemolysis, inflammation and hepcidin activity. *Eur J Appl Physiol* 106: 51-59, 2009.
89. **Pournot H, Bieuzen F, Louis J, Mounier R, Fillard JR, Barbiche E, and Hausswirth C**. Time-course of changes in inflammatory response after whole-body cryotherapy multi exposures following severe exercise. *PLoS One* 6: e22748, 2011.
90. **Prather AA, Hall M, Fury JM, Ross DC, Muldoon MF, Cohen S, and Marsland AL**. Sleep and antibody response to hepatitis B vaccination. *Sleep* 35: 1063-1069, 2012.
91. **Prather AA, Janicki-Deverts D, Hall MH, and Cohen S**. Behaviorally assessed sleep and susceptibility to the common cold. *Sleep* 38: 1353-1359, 2015.
92. **Pruscino CL, Halson S, and Hargreaves M**. Effects of compression garments on recovery following intermittent exercise. *Eur J Appl Physiol* 113: 1585-1596, 2013.
93. **Ricardo JS, Cartner L, Oliver SJ, Laing SJ, Walters R, Bilzon JL, and Walsh NP**. No effect of a 30-h period of sleep deprivation on leukocyte trafficking, neutrophil degranulation and saliva IgA responses to exercise. *Eur J Appl Physiol* 105: 499-504, 2009.
94. **Robson PJ, Blannin AK, Walsh NP, Castell LM, and Gleeson M**. Effects of exercise intensity, duration and recovery on in vitro neutrophil function in male athletes. *Int J Sports Med* 20: 128-135, 1999.
95. **Robson-Ansley PJ, Blannin A, and Gleeson M**. Elevated plasma interleukin-6 levels in trained male triathletes following an acute period of intense interval training. *Eur J Appl Physiol* 99: 353-360, 2007.

96. **Rohde T, MacLean DA, and Pedersen BK**. Effect of glutamine supplementation on changes in the immune system induced by repeated exercise. *Med Sci Sports Exerc* 30: 856-862, 1998.
97. **Ronsen O, Kjeldsen-Kragh J, Haug E, Bahr R, and Pedersen BK**. Recovery time affects immunoendocrine responses to a second bout of endurance exercise. *Am J Physiol Cell Physiol* 283: C1612-1620, 2002.
98. **Ronsen O, Lea T, Bahr R, and Pedersen BK**. Enhanced plasma IL-6 and IL-1ra responses to repeated vs. single bouts of prolonged cycling in elite athletes. *J Appl Physiol* 92: 2547-2553, 2002.
99. **Ronsen O, Pedersen BK, Oritsland TR, Bahr R, and Kjeldsen-Kragh J**. Leukocyte counts and lymphocyte responsiveness associated with repeated bouts of strenuous endurance exercise. *J Appl Physiol* 91: 425-434, 2001.
100. **Rosenberger PH, Ickovics JR, Epel E, Nadler E, Jokl P, Fulkerson JP, Tillie JM, and Dhabhar FS**. Surgical stress-induced immune cell redistribution profiles predict short-term and long-term postsurgical recovery. A prospective study. *J Bone Joint Surg Am* 91: 2783-2794, 2009.
101. **Rowbottom DG, Keast D, Goodman C, and Morton AR**. The haematological, biochemical and immunological profile of athletes suffering from the overtraining syndrome. *Eur J Appl Physiol Occup Physiol* 70: 502-509, 1995.
102. **Severs Y, Brenner I, Shek PN, and Shephard RJ**. Effects of heat and intermittent exercise on leukocyte and sub-population cell counts. *Eur J Appl Physiol Occup Physiol* 74: 234-245, 1996.
103. **Shearer WT, Reuben JM, Mullington JM, Price NJ, Lee BN, Smith EO, Szuba MP, Van Dongen HP, and Dinges DF**. Soluble TNF-alpha receptor 1 and IL-6 plasma levels in humans subjected to the sleep deprivation model of spaceflight. *J Allergy Clin Immunol* 107: 165-170, 2001.
104. **Shek PN, Sabiston BH, Buguet A, and Radomski MW**. Strenuous exercise and immunological changes: a multiple-time-point analysis of leukocyte subsets, CD4/CD8 ratio, immunoglobulin production and NK cell response. *Int J Sports Med* 16: 466-474, 1995.
105. **Simpson R, Florida-James G, Whyte G, Black J, Ross J, and Guy K**. Apoptosis does not contribute to the blood lymphocytopenia observed after intensive and downhill treadmill running in humans. *Res Sports Med* 15: 157-174, 2007.
106. **Simpson RJ**. Aging, persistent viral infections, and immunosenescence: can exercise "make space"? *Exerc Sport Sci Rev* 39: 23-33, 2011.
107. **Simpson RJ, Bigley AB, Spielmann G, LaVoy EC, Kunz H, and Bollard CM**. Human cytomegalovirus infection and the immune response to exercise. *Exerc Immunol Rev* 22: 8-27, 2016.
108. **Simpson RJ, Florida-James GD, Whyte GP, and Guy K**. The effects of intensive, moderate and downhill treadmill running on human blood lymphocytes expressing the adhesion/activation molecules CD54 (ICAM-1), CD18 (beta2 integrin) and CD53. *Eur J Appl Physiol* 97: 109-121, 2006.
109. **Simpson RJ, McFarlin BK, McSporran C, Spielmann G, o Hartaigh B, and Guy K**. Toll-like receptor expression on classic and pro-inflammatory blood monocytes after acute exercise in humans. *Brain Behav Immun* 23: 232-239, 2009.
110. **Sinclair LV, Finlay D, Feijoo C, Cornish GH, Gray A, Ager A, Okkenhaug K, Hagenbeek TJ, Spits H, and Cantrell DA**. Phosphatidylinositol-3-OH kinase and nutrient-sensing mTOR pathways control T lymphocyte trafficking. *Nat Immunol* 9: 513-521, 2008.
111. **Spielmann G, Bollard CM, Bigley AB, Hanley PJ, Blaney JW, LaVoy EC, Pircher H, and Simpson RJ**. The effects of age and latent cytomegalovirus infection on the redeployment of CD8+ T cell subsets in response to acute exercise in humans. *Brain Behav Immun* 39: 142-151, 2014.
112. **Spielmann G, Bollard CM, Kunz H, Hanley PJ, and Simpson RJ**. A single exercise bout enhances the manufacture of viral-specific T-cells from healthy donors: implications for allogeneic adoptive transfer immunotherapy. *Sci Rep* 6: 25852, 2016.
113. **Starkie RL, Angus DJ, Rolland J, Hargreaves M, and Febbraio MA**. Effect of prolonged, submaximal exercise and carbohydrate ingestion on monocyte intracellular cytokine production in humans. *J Physiol* 528: 647-655, 2000.

114. **Steensberg A, Febbraio MA, Osada T, Schjerling P, van Hall G, Saltin B, and Pedersen BK**. Interleukin-6 production in contracting human skeletal muscle is influenced by pre-exercise musde glycogen content. *J Physiol* 537: 633-639, 2001.

115. **Steensberg A, Toft AD, Bruunsgaard H, Sandmand M, Halkjaer-Kristensen J, and Pedersen BK**. Strenuous exercise decreases the percentage of type 1 T cells in the circulation. *J Appl Physiol* 91: 1708-1712, 2001.

116. **Suzuki K, Naganuma S, Totsuka M, Suzuki KJ, Mochizuki M, Shiraishi M, Nakaji S, and Sugawara K**. Effects of exhaustive endurance exercise and its one-week daily repetition on neutrophil count and functional status in untrained men. *Int J Sports Med* 17: 205-212, 1996.

117. **Suzuki K, Nakaji S, Yamada M, Liu Q, Kurakake S, Okamura N, Kumae T, Umeda T, and Sugawara K**. Impact of a competitive marathon race on systemic cytokine and neutrophil responses. *Med Sci Sports Exerc* 35: 348-355, 2003.

118. **Suzuki K, Totsuka M, Nakaji S, Yamada M, Kudoh S, Liu Q, Sugawara K, Yamaya K, and Sato K**. Endurance exercise causes interaction among stress hormones, cytokines, neutrophil dynamics, and muscle damage. *J Appl Physiol* 87: 1360-1367, 1999.

119. **Suzuki M, Umeda T, Nakaji S, Shimoyama T, Mashiko T, and Sugawara K**. Effect of incorporating low intensity exercise into the recovery period after a rugby match. *Br J Sports Med* 38: 436-440, 2004.

120. **Trappe TA, Standley RA, Jemiolo B, Carroll CC, and Trappe SW**. Prostaglandin and myokine involvement in the cyclooxygenase-inhibiting drug enhancement of skeletal muscle adaptations to resistance exercise in older adults. *Am J Physiol Regul Integr Comp Physiol* 304: R198-205, 2013.

121. **Turner JE, Wadley AJ, Aldred S, Fisher JP, Bosch JA, and Campbell JP**. Intensive exercise does not preferentially mobilize skin-homing T cells and NK cells. *Med Sci Sports Exerc* 48: 1285-1293, 2016.

122. **Vella L, Markworth JF, Paulsen G, Raastad T, Peake JM, Snow RJ, Cameron-Smith D, and Russell AP**. Ibuprofen ingestion does not affect markers of post-exercise muscle inflammation. *Front Physiol* 7: 86, 2016.

123. **Vella L, Markworth JF, Peake JM, Snow RJ, Cameron-Smith D, and Russell AP**. Ibuprofen supplementation and its effects on NF-kappaB activation in skeletal muscle following resistance exercise. *Physiol Rep* 2: 2014.

124. **Verde T, Thomas S, and Shephard RJ**. Potential markers of heavy training in highly trained distance runners. *Br J Sports Med* 26: 167-175, 1992.

125. **Walsh NP, Gleeson M, Pyne DB, Nieman DC, Dhabhar FS, Shephard RJ, Oliver SJ, Bermon S, and Kajeniene A**. Position statement. Part two: maintaining immune health. *Exerc Immunol Rev* 17: 64-103, 2011.

126. **Walsh NP, Gleeson M, Shephard RJ, Gleeson M, Woods JA, Bishop NC, Fleshner M, Green C, Pedersen BK, Hoffman-Goetz L, Rogers CJ, Northoff H, Abbasi A, and Simon P**. Position statement. Part one: Immune function and exercise. *Exerc Immunol Rev* 17: 6-63, 2011.

127. **Wigernaes I, Hostmark AT, Stromme SB, Kierulf P, and Birkeland K**. Active recovery and post-exercise white blood cell count, free fatty acids, and hormones in endurance athletes. *Eur J Appl Physiol* 84: 358-366, 2001.

128. **Witard OC, Turner JE, Jackman SR, Kies AK, Jeukendrup AE, Bosch JA, and Tipton KD**. High dietary protein restores overreaching induced impairments in leukocyte trafficking and reduces the incidence of upper respiratory tract infection in elite cyclists. *Brain Behav Immun* 39: 211-219, 2014.

129. **Witard OC, Turner JE, Jackman SR, Tipton KD, Jeukendrup AE, Kies AK, and Bosch JA**. High-intensity training reduces CD8+ T-cell redistribution in response to exercise. *Med Sci Sports Exerc* 44: 1689-1697, 2012.

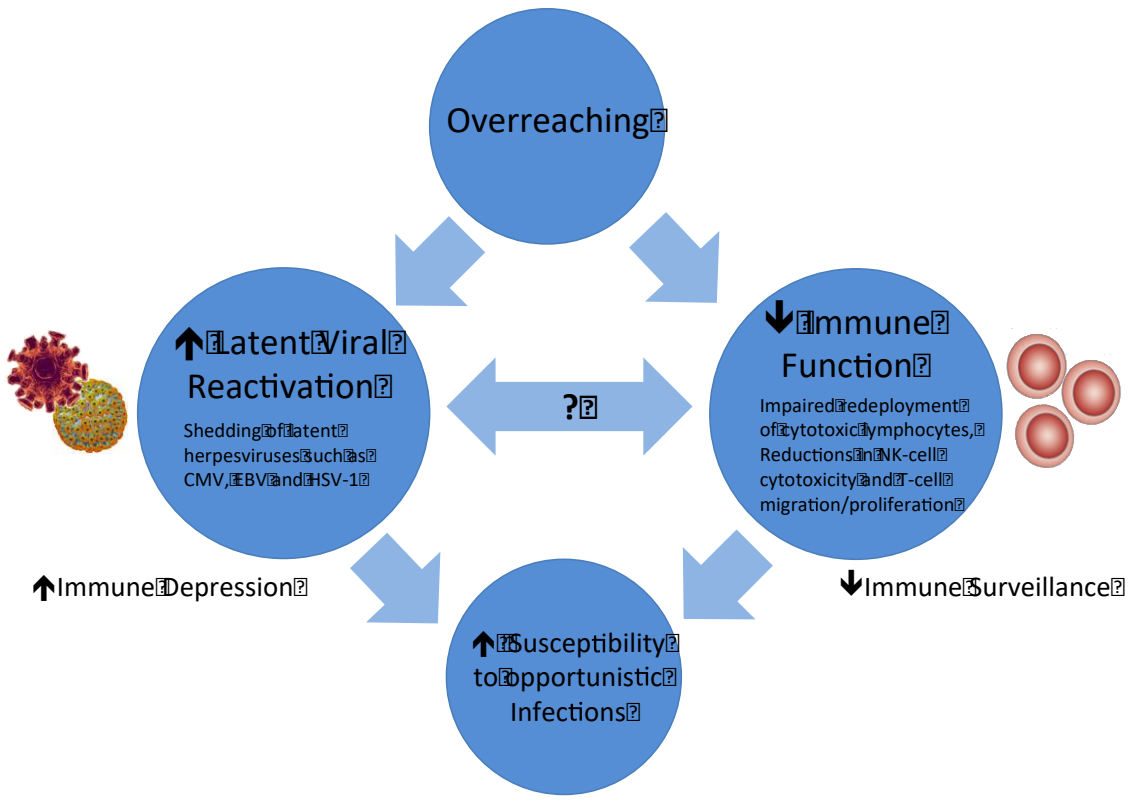
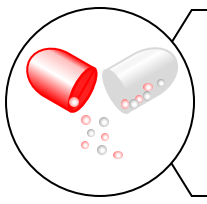
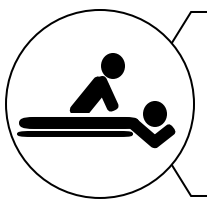


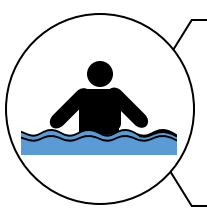
Figure 1. Overreaching or heavy training is associated with impaired cellular immune function and latent viral reactivation. Reduction in lymphocyte trafficking and function during exercise recovery impairs immune surveillance, which may increase susceptibility to opportunistic infection. Lowered immunity may also allow previously acquired viruses to reactivate from a latent state, which may cause further immunodepression and increase susceptibility to infection.



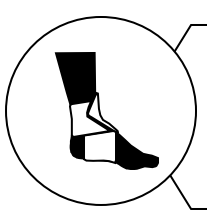
Non-steroidal anti-inflammatory drugs:
- no Δ inflammatory cells, cytokine mRNA expression, NFκB binding activity in muscle
- IL-6 release from tendons



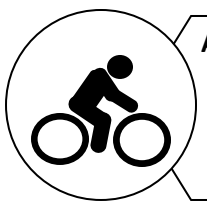
Massage: ↓ NFκB (p65) accumulation, IL-6 and TNF-α protein expression in muscle
Vibration therapy: ↑ blood neutrophils, ↓ blood lymphocytes and plasma IL-6 concentration
Electrical stimulation: no Δ plasma IL-6 concentration



Cold water immersion: ↓ blood neutrophils and monocytes; no Δ plasma IL-1β, IL-6 and IL-10
Icing: ↓ plasma IL-1β, IL-1ra, IL-6 and TNF-α
Cryotherapy: ↓ plasma IL-1β; ↑ plasma IL-1ra



Compression garments: no Δ plasma IL-1β, IL-6 and TNF-α



Active recovery:
- ↑ blood neutrophils; ↓ blood lymphocytes
- no Δ plasma cytokines, C-reactive protein, complement

Figure 2. Summary of the effects of nonsteroidal anti-inflammatory drugs (NSAIDs) and physical therapies on immune changes during recovery from exercise.

Table 1. Evidence heatmap comparing differences in immune responses to two versus one exercise bout on the same day (A), short vs long recovery between bouts on the same day (B), consecutive days of exercise (C), and weeks (D) or months (E) of intensified training.

	A			B		C		D		E	
Leukocyte cell count	5	3				2		1		2	
Neutrophil cell count	8	3		2		2	1	2		2	
Monocyte cell count	3	2			1			2		2	1
Lymphocyte cell count	4	3	2		1		1	2		2	
Eosinophil cell count			1		1					1	
Oxidative burst activity			1		1	1	2				
Oxidative burst activity/neutrophil	1			1							
Plasma ELA concentration							1				
Plasma MPO concentration						1					
LPS-induced ELA release			1					1			
LPS-induced ELA release/neutrophil	1										
$CD3^+$ T cell count	1		1			1				2	
$CD4^+$ T cell count	4		2		1					2	
$CD8^+$ T cell count	4	1	1	1						2	
CD4:CD8 ratio								1	1		
$CD19^+$ B cell count	1	1								2	
$CD4^+/CD69^+$ T cell count			1		1						
$CD8^+/CD69^+$ T cell count			1	1							
Lymphocyte proliferation			2			1			1		
Antibody production		1							1		
Saliva IgA concentration	1	1						2			5
Saliva IgA secretion rate	1	1				1	1				1
$CD16^+$ or $CD56^+$ NK cell count	2	2		1			1		1	2	
$CD56^+/CD69^+$ NK cell count	1				1						
NK cell cytotoxicity		1	1				1				
LPS-induced IL-6 production			1								
LPS-induced IL-8 production	1										
Leukocyte IL-8 mRNA							1				
Leukocyte IL-10 mRNA							1				
Leukocyte IL-1ra mRNA						1					
Plasma IL-6 concentration					1		2	1	1		
Plasma IL-1ra concentration					1		1				
Plasma IL-8 concentration							1				
Plasma IL-10 concentration							1				
Plasma TNF-α concentration							1		1		
Plasma MCP-1 concentration							1				
Muscle IL-6 mRNA expression							1				
Muscle IL-8 mRNA expression							1				
Muscle IL-1β mRNA expression							1				
Muscle TNF-α mRNA expression							1				

Numbers represent the number of studies demonstrating an increase/greater change (red), no difference (green), or decrease/smaller change (blue) compared with the first bout of exercise, long recovery, before training, or healthy athletes (refer to text). Abbreviations: ELA, elastase; MPO, myeloperoxidase; NK, natural killer; MCP-1, monocyte chemoattractant protein 1

831

832

833